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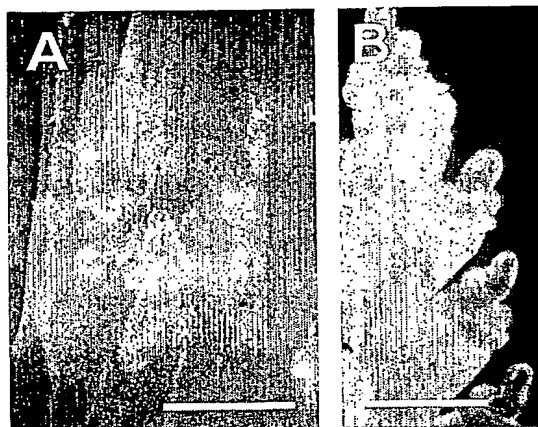
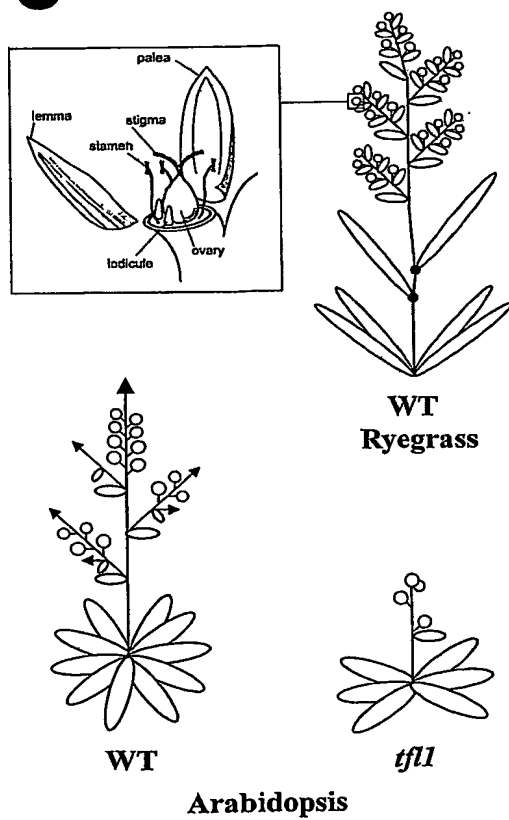
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- (74) Agents: **MACDOUGALL, Donald, Carmichael et al.**; Cruikshank & Fairweather, 19 Royal Exchange Square, Glasgow G1 3AE (GB).
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- (71) Applicants (*for all designated States except US*): **DLF - TRIFOLIUM A/S** [DK/DK]; Hoejerupvej, P.O. Box 19, DK-4660 Store Hedding (DK). **RISØE NATIONAL LABORATORY** [DK/DK]; P.O. Box 49, DK-4000 Roskilde (DK).
- (72) Inventors; and
- (75) Inventors/Applicants (*for US only*): **NIELSEN, Klaus, K.** [DK/DK]; Nebbegaardsbakken 9, DK-2400 Copenhagen NV (DK). **JENSEN, Christian, Sig** [DK/DK]; Moltkesvej 61, 2th, DK-2000 Frederiksberg (DK). **GAO, Caixa** [DK/DK]; Maglekaeret 37B, DK-2680 Solroed Strand (DK). **SALCHERT, Klaus** [DE/DE]; Steinberg Strasse 16, 06507 Gernrode (DE).
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(54) Title: METHOD OF REPRESSING FLOWERING IN A PLANT

(57) Abstract: The isolation and function of a plant *LpTFL1* from *Lolium perenne* (perennial ryegrass) are described, along with generation of transgenic *Arabidopsis*, ryegrass, and red fescue plants. The gene prevents or represses flowering of transgenic plants. Methods for using the gene to repress or prevent flowering are described.

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**FIGURE 1****C**

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**FIGURE 2**

		GCC	-76
-75	CAAGCCACTTCAAAGCTTTGCTACTACCAGATAGAGCATTACCGTGCAATATAGAAATACTTGCCTCTCCAACC		-1
1	ATGTCTAGGTCTGTGGAGCCTCTTATTGTTGGTCGTGTTCATTGGAGAAGTTCTCGATCCATTTAACCCATGTGTG		75
76	AAGATGGTAGCAACCTATAACTCAAACAAGCTGGTCTTCAATGGTCATGAGCTCTACCCATCAGCAGTTGTATCT		150
151	AAACCAAGAGTAGAGGTTGAGGGGGGTGACTTGCGATCCTTATTCACATTGGTTATGACGGACCCAGATGTGCCA		225
226	GGACCAAGTGATCCGTATCTGCGGGAGCATCTTCACTGGATTGTCAGTAATATACCTGGGACAACAGATGCTTCA		300
301	TTTGGGGGGGAGGTCATGAGCTATGAGAGCCCAAAGCCCAACATTGGAATCCACAGGTTCATTTTTGTGCTCTTC		375
375	AAGCAGAAGCGAAGGCAGACTGTATCTGTGCCTTCCTTCAGGGATCATTTCAACACCCGCCAGTTTGCTGTGGAT		450
451	AATGATCTTGGCCTCCCTGTGGCTGCTGTTTACTTCAATTGTGAGAGAGAGACTGCTGCCAGGAGGCGCTGAAAA		525
526	TCGAGTTCTTGGCTATCCAGTTGTGCCAAATAAAGGCTTTTGGAGTTATGCACCTTCTTTCTGAAGTCAATGCT		600
601	CCTCTTCTACATTACTTCCTCGTGGACCATTGCTTCTTTACTACAGTTTTTGCTCAGGGATCAAATAAATCAAGT		675
675	GCATTTTGGAGATTGTATTAGATTATATTGTAAGCAGTGAGATCAGCAACCATGTGTTAACATAAGCCAGTACAT		750
751	TAGCAGGTCCATGTTTATGGTTTCATGTTGTGTGTAAGCAGTTATCACTAGAAGGAAGGTCAGGTAGACAACCCA		825
826	AACTGGCAAAAAAAGCTTTATCTA		851

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**FIGURE 3**

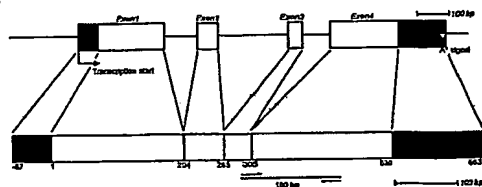
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-3450 aagcgatccaaagggtgctgggttaacgggtatgacagcaaacagaaaacatcgccatctgcacggaagccagaagt -3376  
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-3150 atgattcaatagaagatgcatgtgccattacagagtggattattatgtcctttttaagagatgcttactgcctc -3076  
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-3000 aatactatagaacttccctaatttaatttccccttattttctggactctatcttaattctcctcctattgttcag -2926  
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-2850 cctgaatctatttctcactcatgctgcaatgctccttctcacagcaaatatggatgatctgcagtaagctc -2776  
-2775 aaccttctgcatgtatgccagtggcaacgcgagttcagcatttgggtcgcgcgagctgccccaacgctcaa -2701  
-2700 ccagccctgcagaagggtgctaaatccatcatcatccttactctctggagattatggaagacgaggaacgatgct -2626  
-2625 atcttcaaaaactctggccccaacagactgccttagttcagtcgatcctagatgaagcctgtcaatggctgta -2551  
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-1875 ctactgtgctaccaacacactgtagcactgccaaaaatttatgaaaaagctgaaacagacgagatgtatctatca -1801  
-1800 attcatggaccattttgttataattttcttttaaaaaataaaaaatccgtaaaagaatcaataagtgaattattg -1726  
-1725 gaaatgaaaaaagtaacaaaaataactaaacttttttcaaatacagatcggtatcatggagacacactggctac -1651  
-1650 cattggttgaatagctactagattccactacagctaggtgtcagcaactataatggcatcagaatggagcaga -1576  
-1575 aaaaatgtcacagctgtacttcaactccactacttctagctgcacaaatgtcaagcaggcatgattgcactagacc -1501  
-1500 agaacatagtaatgcataaagctgtaattggctccactacttatggaaacgaagaaatctattatttattgtttt -1426  
-1425 aatcgagatgaagctgtgataattttatcgctgaaatgacatttcagcactagacagaccctagacaattaaagt -1351  
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-1200 acaacttcccttttcccttaacagaaaaagaatcggtcgaacgaggttgcctaaaccaacacactataaagacg -1126  
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-975 caaaaacgaattgatagtttaggaaagcatcactccaaagtgttttattcccggttcttttctattgtctccacaa -901  
-900 gggcatattcctaaatttctgcgaacaattacatctagatctttttaaactgaagtatttttagcatgaaaacg -826  
-825 cattgttctgtaattgtggctgtgaatttcggactgctcatctgatttccctctggtagaatacataaataattat -751  
-750 acacaacagcatgataatgtgcaaaactaagcatcaaaatctgcacattgtcatgcagaaactaggacaggagga -676  
-675 ccagcactttgtcgtttgtcctaaccaatattaacatagttcagcaacataatcttcagagaccactagcatga -601  
-600 aggtgtgttatgtttcctaagaataaacatgtaggtagtgatctacaatacctttttggggactataaggtgg -526  
-525 gaaaccatcaacttgaaggtttccatttaataagtaaaaaaacagattttttaactatcaataactaaaa -451  
-450 ttaaaacagaatagatataactaacaatgaaaatcaaacagttgtgcaaatgtatttatcgtagttagtatct -376  
-375 catgtttctggtgaaaaaatttctctgccctagaacttggagaagatgcatgaagtattactccaaactccaac -301  
-300 actgtgcaactgatagaaaagaacagacccttgggtggctgtctcgaaaaagtggttaggtcctttctgtgg -226  
-225 ccttttcagttctttccacgcataaccaacaaaaagaacacagataactactcatgtctcacattctcttttga -151  
-150 gcttactactcgaagcaggtcttctgcctctataagtagaggctcgtcgtactctagcaatgctcagtaagcaGCC -76  
-75 CAAGCCACTTCAAAGCTTTGCTACTACAGATAGAGCATTCACCGTGCAATATAGAAATACTTGCCCTCTCCAACC -1  
1 ATGCTCTAGGCTCTGTGGAGCCTCTTATTGTTGGTCTGTCTATTGGAGAAGTTCTCGATCCATTTAAGCCATGTGTG 75  
76 AAGATGGTAGCAACCTATAACTCAAACAAGCTGGTCTTCAATGGTCAAGCTCTACCCATCAGCAGTTGTATCT 150  
151 AAACCAAGTAGAGGTTTCAAGGGGGGTGACTTGCGATCCTTATTACATTGGtagaatgcactcgactcgatctt 225  
226 ggaactcatattcaacttcgagattgtatgcttgttttctcttctcgcagtgccataattattcatatttca 300  
301 gGTTATGACGGACCCAGATGTGCCAGGACCAAGTGATCCGTATCTGCGGGAGCATCTTCACTGGtaacctttctc 375  
375 atgcacagtttttctgctgggtggctactaagcacctaaatatattagatatattttttgaaaggaaaatatat 450

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451	tagtatatgttgctaaggaatatagaagtacatcttcttcttgccatatatagacagagagactattttaataag	525
526	cacttctaacgagagtcattttaccaataccttttacacttacacaggATTGTCAGTAATATACCTGGGACAACAG	600
601	ATGCTTCATTGGtaggtccttctctgagatttgaattgggtatattctatgttctgcattttgaatgaataacca	675
675	ctgaccttttgaattgcaggGGGGGAGGTCATGAGCTATGAGAGCCCCAAGCCCAACATTGGAATCCACAGGTTTC	750
751	ATTTTGTGCTCTTCAAGCAGAAGCGAAGGCAGACTGTATCTGTGCCCTTCCTTCAGGGATCATTTCAACACCCGC	825
826	CAGTTTGTGCTGGATAATGATCTTGGCCTCCCTGTGGCTGCTGTTTACTTCAATTGTCAGAGAGAGACTGCTGCC	900
901	AGGAGGCGCTGAAAATCGAGTTCTTGGCTATCCAGTTGTGCCAAATAAAGGCTTTTGGAGTTATGCACCTTCTT	975
976	TCTGAAGTCAATGCTCCTCTTCTACATTACTTCCTCGTGGACCATTTGCTTCTTTACTACAGTTTTTGTCTCAGGGA	1050
1051	TCAAATAAATCAAGTGCATTTTGGAGATTGTATTAGATTATATTGTAAGCAGTGAGATCAGCAACCATGTGTAA	1125
1126	CATAAGCCAGTACATTAGCAGGTCATGTTTATGGTTTCATGTTGTGTGTAAGCAGTTATCACTAGAAGGAAGGT	1200
1201	CAGGTAGACAACCCAAACTGGCAAAAAAAAAAGCTTTATCTActgtatggcccttgccggttgatgttccatgc	1275
1276	accttttctgacatgctgtctactgtatgccaccgccactataatgtatgagatatgaatataaaatggagatat	1350
1351	ccaaaatatccagatgattgccactaaatgctaaatgtacatagtggttttccacctattttgacttcatcat	1425
1426	gtccttacacaaaatcagaaaacatccatttcatgcacattgatgcacactgcatattaacaatctattcagatt	1500
1501	tggctgtaaacacacccttattttccgcatccattaatattatattagtagtaccctggacaggttaagcttttgcag	1575
1576	cacagtaagtaaccggatgaaattacaatatgatcctcgagcgccctat	1624

**FIGURE 4**

1	MSRSVEPLIVGRVIGEVLDPFNPCKMVAATYNSNKLVFNGHELYPSAVVSKPRVEVQGGDLRLSLFTLVMTDPDVP	75
76	GPSDPYLREHLHWIVSNIPGTTDASFGGEVMSYESPKPNIGIHRFIFVLFKQRRQTVSVPSFRDHFNTNRQFAVD	150
151	NDLGLPVAAYVFNCRQRETAARRR	173

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**FIGURE 5****A****B**

TFL1	M	N	M	G	R	V	I	E	P	L	M	G	R	V	V	C	D	V	L	D	F	T	F	T	K	H	N	V	S	H	K	37
BNTFL1-1	M	N	M	G	R	V	I	E	P	L	V	G	R	V	V	C	D	V	L	D	F	T	F	T	K	H	N	V	S	H	K	37
BNTFL1-3	M	N	M	G	R	V	I	E	P	L	V	G	R	V	V	C	D	V	L	D	F	T	F	T	K	H	N	V	S	H	K	37
CEN	M	-	-	A	A	K	V	S	S	D	P	L	V	G	R	V	C	D	V	L	D	F	T	S	V	K	S	V	X	H	S	35
CET2	M	-	-	G	S	K	M	S	-	D	P	L	V	G	R	V	C	D	V	L	D	F	T	S	V	K	S	V	X	H	S	34
CET4	M	-	-	G	S	K	M	S	-	D	P	L	V	G	R	V	C	D	V	L	D	F	T	S	V	K	S	V	X	H	S	34
SP	M	-	-	A	A	K	V	S	S	D	P	L	V	G	R	V	C	D	V	L	D	F	T	S	V	K	S	V	X	H	S	34
CET1	M	-	-	A	A	K	V	S	S	D	P	L	V	G	R	V	C	D	V	L	D	F	T	S	V	K	S	V	X	H	S	34
LpTFL1	M	-	-	S	R	S	V	-	-	E	P	L	V	G	R	V	C	D	V	L	D	F	T	S	V	K	S	V	X	H	S	33
FDR2	M	-	-	S	R	S	V	-	-	E	P	L	V	G	R	V	C	D	V	L	D	F	T	S	V	K	S	V	X	H	S	33
FDR1	M	-	-	S	R	S	V	-	-	E	P	L	V	G	R	V	C	D	V	L	D	F	T	S	V	K	S	V	X	H	S	33
FT	M	-	-	S	R	S	V	-	-	E	P	L	V	G	R	V	C	D	V	L	D	F	T	S	V	K	S	V	X	H	S	34

TFL1	-	-	-	K	Q	V	S	H	G	H	E	L	F	P	S	A	V	S	K	P	R	V	E	S	G	G	L	R	S	F	F	T	L	V	71
BNTFL1-1	-	-	-	K	Q	V	S	H	G	H	E	L	F	P	S	A	V	S	K	P	R	V	E	S	G	G	L	R	S	F	F	T	L	V	71
BNTFL1-3	-	-	-	K	Q	V	S	H	G	H	E	L	F	P	S	A	V	S	K	P	R	V	E	S	G	G	L	R	S	F	F	T	L	V	71
CEN	M	N	S	K	M	V	X	H	G	H	E	L	F	P	S	A	V	S	K	P	R	V	E	S	G	G	L	R	S	F	F	T	L	V	73
CET2	S	-	-	K	Q	V	S	H	G	H	E	L	F	P	S	A	V	S	K	P	R	V	E	S	G	G	L	R	S	F	F	T	L	V	69
CET4	S	-	-	K	Q	V	S	H	G	H	E	L	F	P	S	A	V	S	K	P	R	V	E	S	G	G	L	R	S	F	F	T	L	V	69
SP	M	-	-	K	Q	V	S	H	G	H	E	L	F	P	S	A	V	S	K	P	R	V	E	S	G	G	L	R	S	F	F	T	L	V	69
CET1	S	-	-	K	Q	V	S	H	G	H	E	L	F	P	S	A	V	S	K	P	R	V	E	S	G	G	L	R	S	F	F	T	L	V	69
LpTFL1	N	-	-	K	Q	V	S	H	G	H	E	L	F	P	S	A	V	S	K	P	R	V	E	S	G	G	L	R	S	F	F	T	L	V	68
FDR2	N	-	-	K	Q	V	S	H	G	H	E	L	F	P	S	A	V	S	K	P	R	V	E	S	G	G	L	R	S	F	F	T	L	V	68
FDR1	N	-	-	K	Q	V	S	H	G	H	E	L	F	P	S	A	V	S	K	P	R	V	E	S	G	G	L	R	S	F	F	T	L	V	68
FT	N	-	-	K	Q	V	S	H	G	H	E	L	F	P	S	A	V	S	K	P	R	V	E	S	G	G	L	R	S	F	F	T	L	V	69

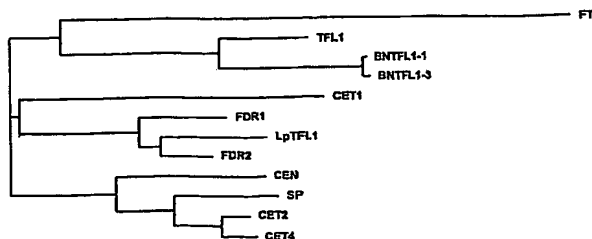
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BNTFL1-1	M	C	D	P	D	V	F	G	P	S	D	F	L	R	E	H	L	H	W	I	V	T	D	I	P	G	T	D	A	S	F	G	R	E	V	109
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CEN	M	C	D	P	D	V	F	G	P	S	D	F	L	R	E	H	L	H	W	I	V	T	D	I	P	G	T	D	A	S	F	G	R	E	V	111
CET2	M	C	D	P	D	V	F	G	P	S	D	F	L	R	E	H	L	H	W	I	V	T	D	I	P	G	T	D	A	S	F	G	R	E	V	107
CET4	M	C	D	P	D	V	F	G	P	S	D	F	L	R	E	H	L	H	W	I	V	T	D	I	P	G	T	D	A	S	F	G	R	E	V	107
SP	M	C	D	P	D	V	F	G	P	S	D	F	L	R	E	H	L	H	W	I	V	T	D	I	P	G	T	D	A	S	F	G	R	E	V	107
CET1	M	C	D	P	D	V	F	G	P	S	D	F	L	R	E	H	L	H	W	I	V	T	D	I	P	G	T	D	A	S	F	G	R	E	V	107
LpTFL1	M	C	D	P	D	V	F	G	P	S	D	F	L	R	E	H	L	H	W	I	V	T	D	I	P	G	T	D	A	S	F	G	R	E	V	106
FDR2	M	C	D	P	D	V	F	G	P	S	D	F	L	R	E	H	L	H	W	I	V	T	D	I	P	G	T	D	A	S	F	G	R	E	V	106
FDR1	M	C	D	P	D	V	F	G	P	S	D	F	L	R	E	H	L	H	W	I	V	T	D	I	P	G	T	D	A	S	F	G	R	E	V	106
FT	M	C	D	P	D	V	F	G	P	S	D	F	L	R	E	H	L	H	W	I	V	T	D	I	P	G	T	D	A	S	F	G	R	E	V	106

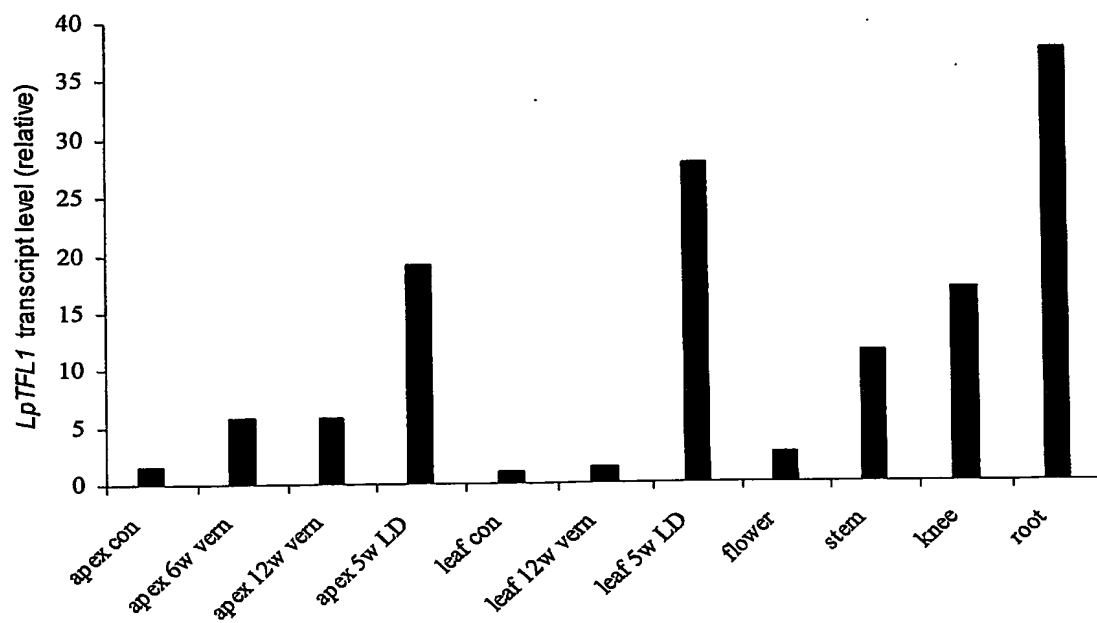
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BNTFL1-1	S	V	E	S	T	K	K	H	I	G	H	R	A	P	I	E	V	L	F	K	Q	K	R	-	R	V	I	F	-	H	I	P	R	D	H	146
BNTFL1-3	S	V	E	S	T	K	K	H	I	G	H	R	A	P	I	E	V	L	F	K	Q	K	R	-	R	V	I	F	-	H	I	P	R	D	H	146
CEN	S	V	E	S	T	K	K	H	I	G	H	R	A	P	I	E	V	L	F	K	Q	K	R	-	R	V	I	F	-	H	I	P	R	D	H	149
CET2	G	Y	E	S	T	K	K	H	I	G	H	R	A	P	I	E	V	L	F	K	Q	K	R	-	R	V	I	F	-	H	I	P	R	D	H	143
CET4	G	Y	E	S	T	K	K	H	I	G	H	R	A	P	I	E	V	L	F	K	Q	K	R	-	R	V	I	F	-	H	I	P	R	D	H	143
SP	G	Y	E	S	T	K	K	H	I	G	H	R	A	P	I	E	V	L	F	K	Q	K	R	-	R	V	I	F	-	H	I	P	R	D	H	143
CET1	S	V	E	S	T	K	K	H	I	G	H	R	A	P	I	E	V	L	F	K	Q	K	R	-	R	V	I	F	-	H	I	P	R	D	H	142
LpTFL1	S	V	E	S	T	K	K	H	I	G	H	R	A	P	I	E	V	L	F	K	Q	K	R	-	R	V	I	F	-	H	I	P	R	D	H	141
FDR2	S	V	E	S	T	K	K	H	I	G	H	R	A	P	I	E	V	L	F	K	Q	K	R	-	R	V	I	F	-	H	I	P	R	D	H	141
FDR1	S	V	E	S	T	K	K	H	I	G	H	R	A	P	I	E	V	L	F	K	Q	K	R	-	R	V	I	F	-	H	I	P	R	D	H	141
FT	S	V	E	S	T	K	K	H	I	G	H	R	A	P	I	E	V	L	F	K	Q	K	R	-	R	V	I	F	-	H	I	P	R	D	H	141

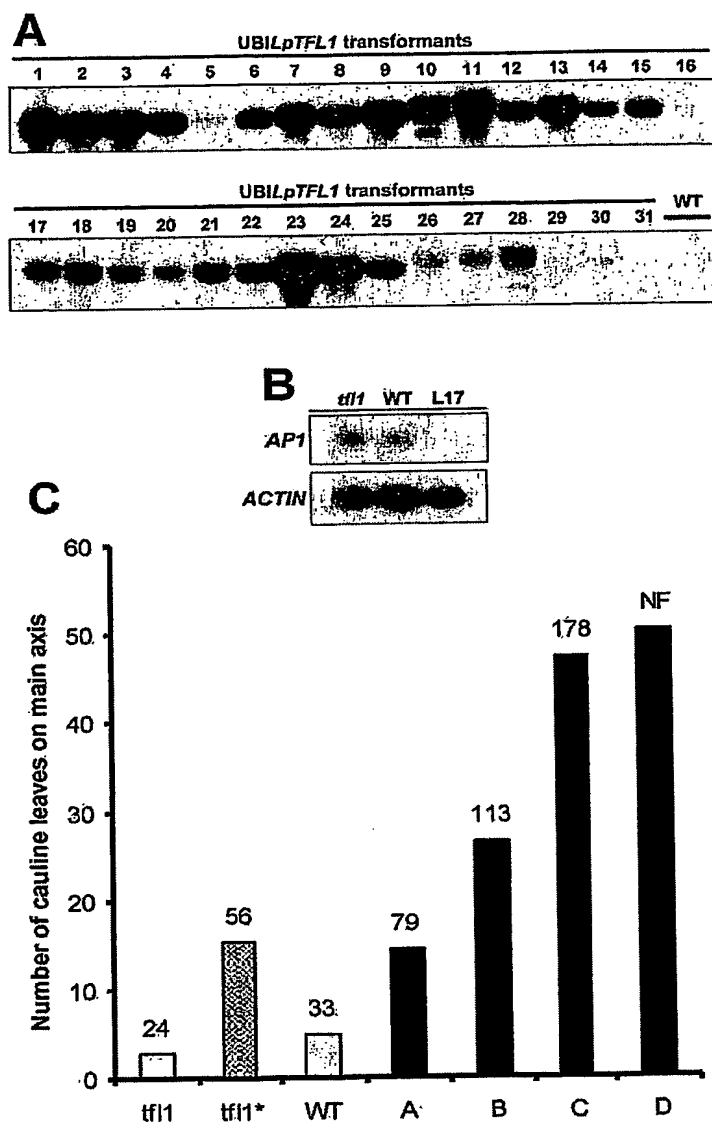
TFL1	F	H	T	R	K	F	A	E	H	D	L	G	L	P	V	A	A	V	F	F	H	A	Q	R	E	T	A	-	-	A	R	R	R	177
BNTFL1-1	F	H	T	R	K	F	A	E	H	D	L	G	L	P	V	A	A	V	F	F	H	A	Q	R	E	T	A	-	-	A	R	R	R	178
BNTFL1-3	F	H	T	R	K	F	A	E	H	D	L	G	L	P	V	A	A	V	F	F	H	A	Q	R	E	T	A	-	-	A	R	R	R	178
CEN	F	H	T	R	K	F	A	E	H	D	L	G	L	P	V	A	A	V	F	F	H	A	Q	R	E	T	A	-	-	A	R	R	R	181
CET2	F	H	T	R	K	F	A	E	H	D	L	G	L	P	V	A	A	V	F	F	H	A	Q	R	E	T	A	-	-	A	R	R	R	175
CET4	F	H	T	R	K	F	A	E	H	D	L	G	L	P	V	A	A	V	F	F	H	A	Q	R	E	T	A	-	-	A	R	R	R	175
SP	F	H	T	R	K	F	A	E	H	D	L	G	L	P	V	A	A	V	F	F	H	A	Q	R	E	T	A	-	-	A	R	R	R	175
CET1	F	H	T	R	K	F	A	E	H	D	L	G	L	P	V	A	A	V	F	F	H	A	Q	R	E	T	A	-	-	A	R	R	R	174
LpTFL1	F	H	T	R	K	F	A	E	H	D	L	G	L	P	V	A	A	V	F	F	H	A	Q	R	E	T	A	-	-	A	R	R	R	173
FDR2	F	H	T	R	K	F	A	E	H	D	L	G	L	P	V	A	A	V	F	F	H	A	Q	R	E	T	A	-	-	A	R	R	R	173
FDR1	F	H	T	R	K	F	A	E	H	D	L	G	L	P	V	A	A	V	F	F	H	A	Q	R	E	T	A	-	-	A	R	R	R	173
FT	F	H	T	R	K	F	A	E	H	D	L	G	L	P	V	A	A	V	F	F	H	A	Q	R	E	T	A	-	-	A	R	R	R	175

**C**

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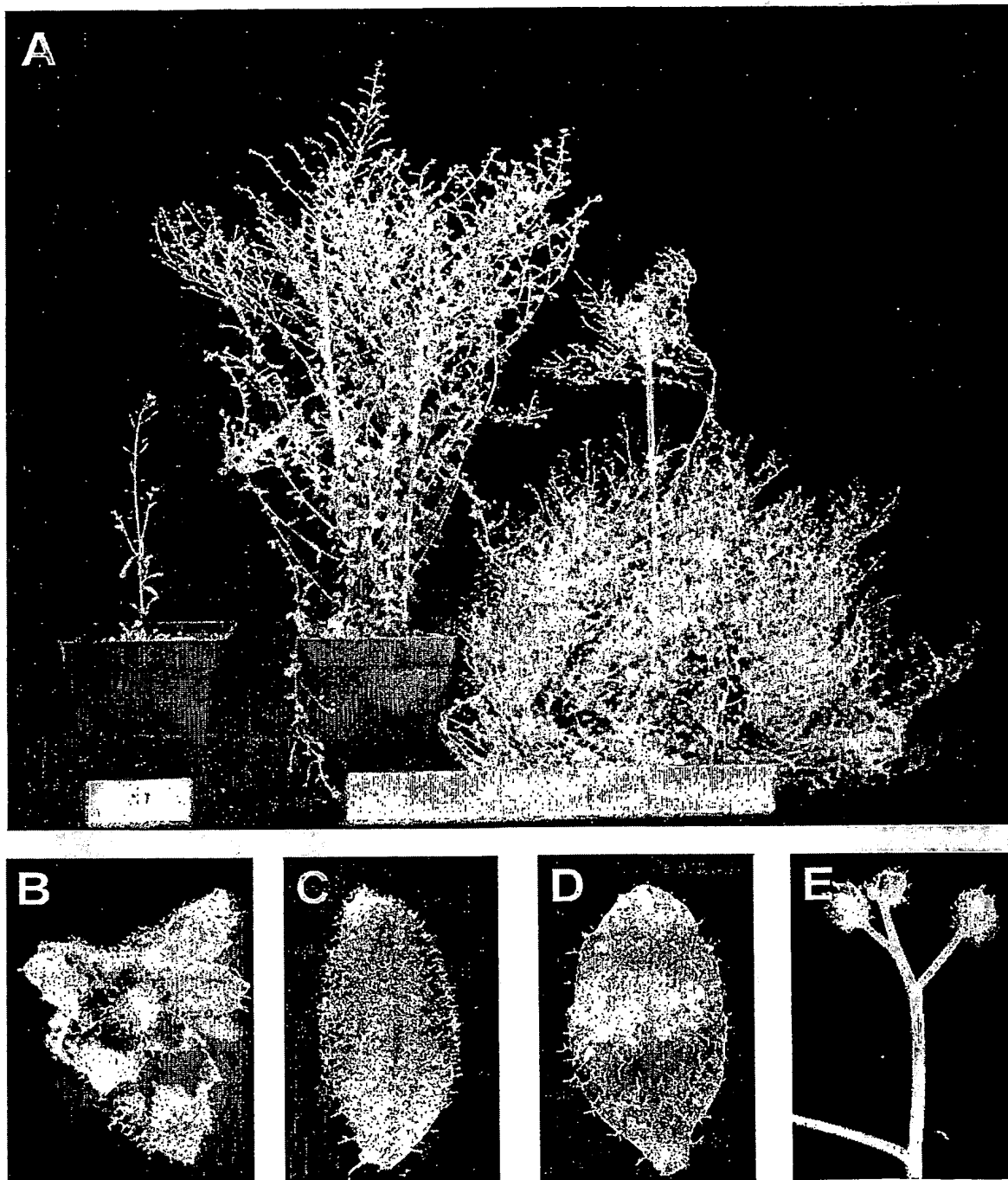
**FIGURE 6**

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**FIGURE 7**

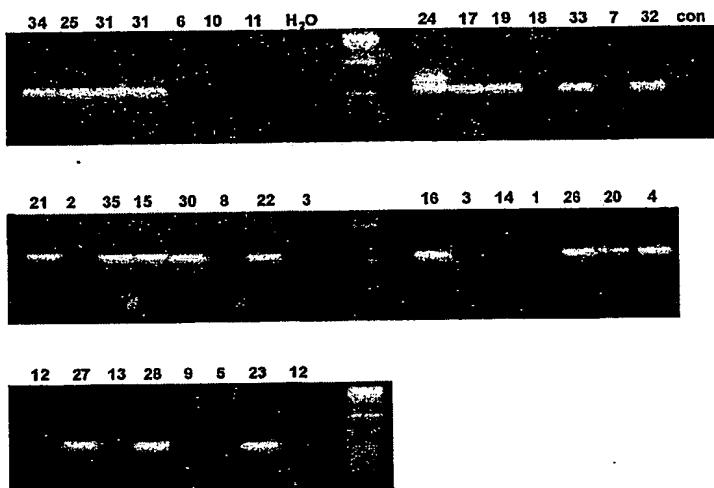


**FIGURE 8**



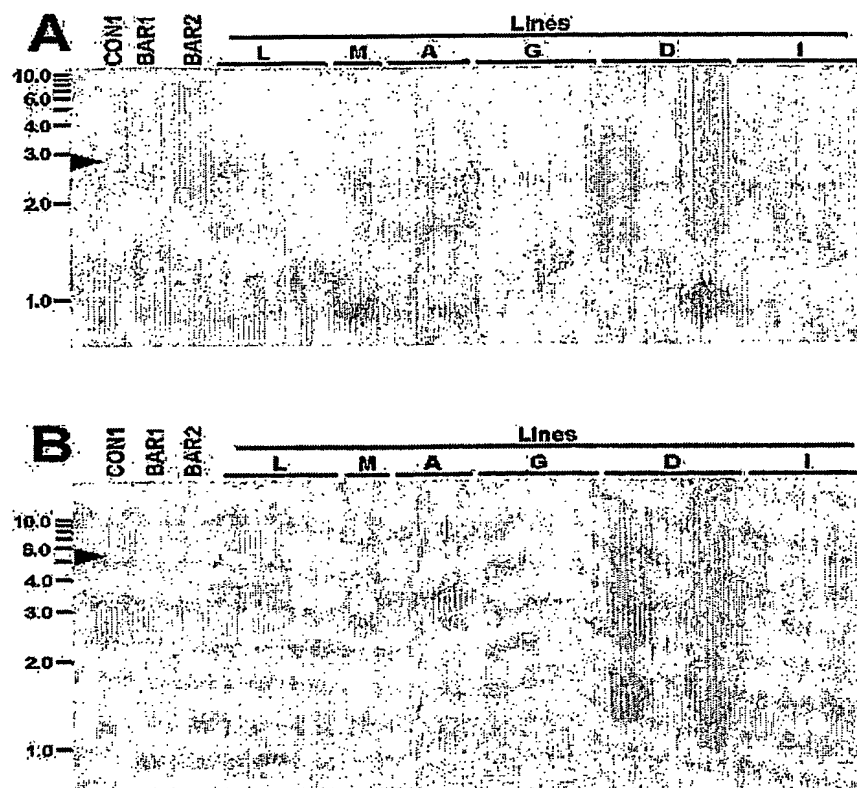
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**FIGURE 9**

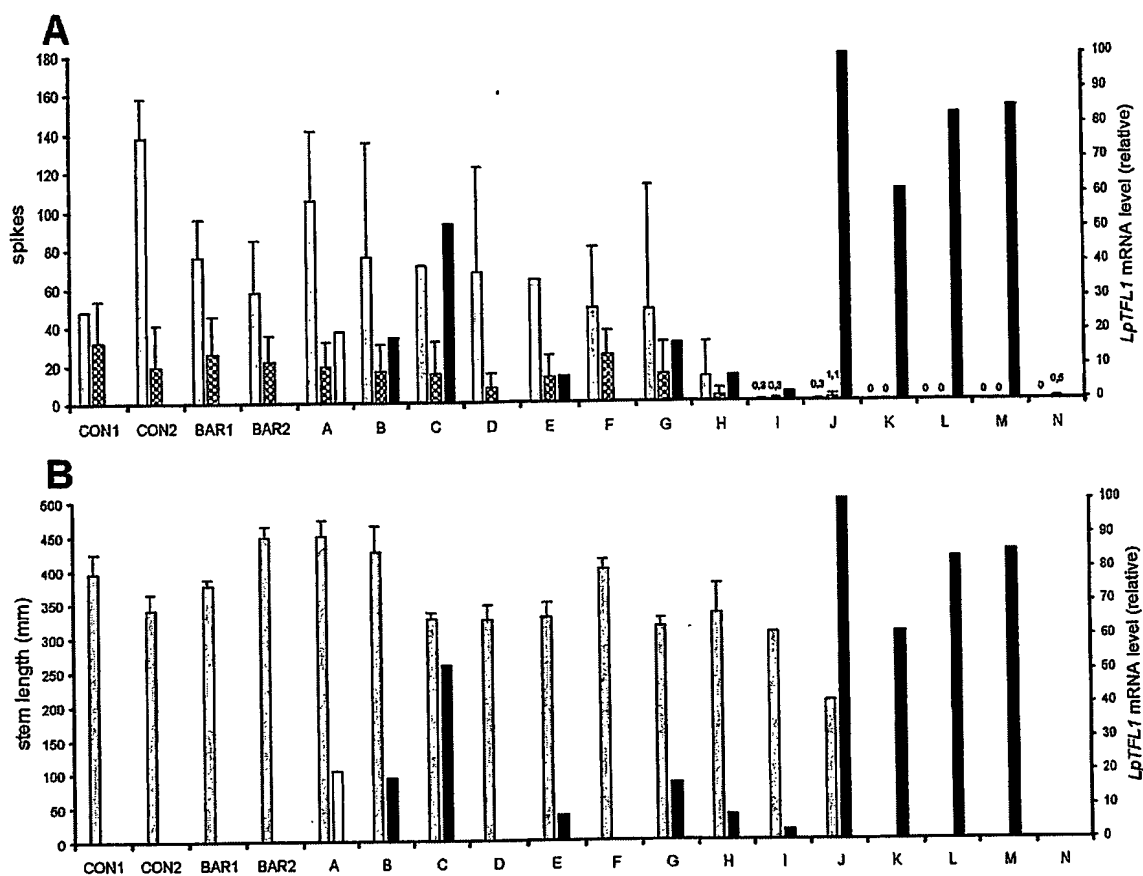
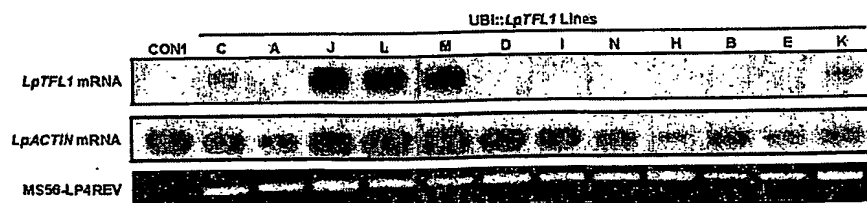


**FIGURE 10**

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**FIGURE 11**

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**FIGURE 12****FIGURE 13**

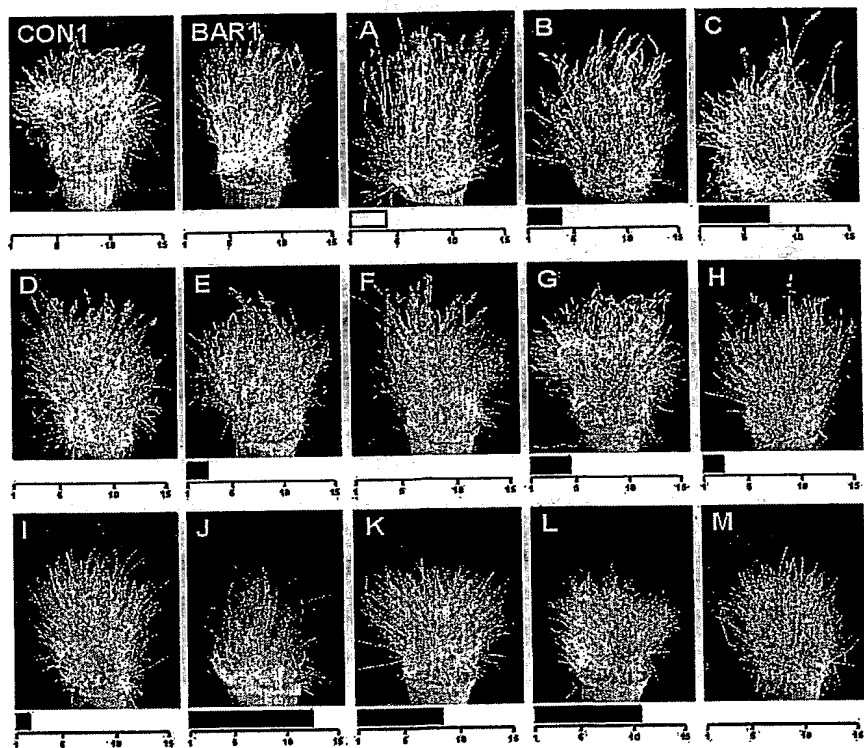
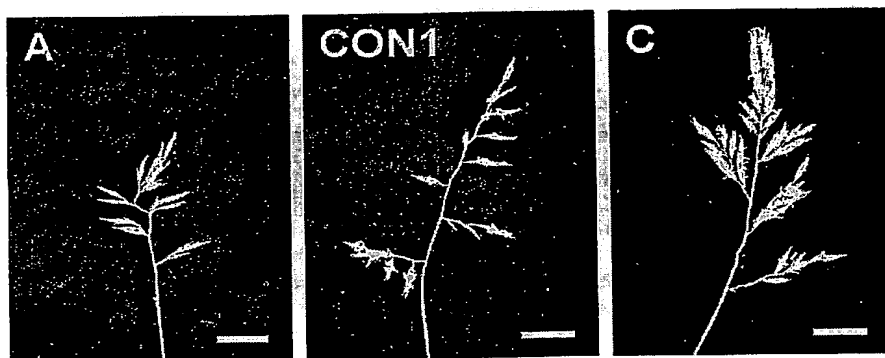
**FIGURE 14****FIGURE 15**

FIGURE 16: *Transformation Efficiency and Floral Activity of the Transformants*

Cultivar	Line No.	Inflorescences	PCR	RT-PCR
F6	CON	8	-	-
F6	7	18	-	-
F6	8	11	-	-
F6	17	5,3	+	-
F6	18	13,3	+	-
F6	24	12	+	+
F6	29	0	+	+
F6	32	0	+	+
F6	33	4	+	+
F6	36	0	+	+
ACTION	2	1,8	-	-
ACTION	5	3	-	-
ACTION	9	0,3	-	-
ACTION	12	2	-	-
ACTION	13	0	-	-
ACTION	16	0	+	-
ACTION	19	7,3	+	-
ACTION	21	4	+	+
ACTION	22	0,3	+	+
ACTION	23	0	+	+
ACTION	25	0,3	+	+
ACTION	27	0	+	+
ACTION	28	4	+	+
ACTION	31	0	+	+
ACTION	34	0	+	+
ACTION	35	0	+	+
TELSTAR	1	10	-	-
TELSTAR	3	1	-	-
TELSTAR	4	11,6	-	-
TELSTAR	6	10,8	-	-
TELSTAR	10	5	-	-
TELSTAR	11	3,8	-	-
TELSTAR	14	0	-	-
TELSTAR	15	3,8	+	-
TELSTAR	20	3,5	+	-
TELSTAR	26	0	+	+
TELSTAR	30	3,7	+	+

Figur 17: Transgene integration analysis by PCR using different primer combinations

Primer combination	UBI::LpTFL1 transgenic lines <sup>a</sup>																			
	CASSETTE	CON	BAR	A	B	C	D	E	F	G	H	I	J	K	L	M	N	P		

<sup>a</sup>plus indicates that the observed fragment had the expected size, whereas numbers indicate that the fragment size deviated from the expected size (numbers in bold), blank field indicates that no PCR-product was detected; E, EcoRI; H, HindIII